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(FILE 'HOME' ENTERED AT 13:26:36 ON 31 MAY 2006)

FILE 'STNGUIDE' ENTERED AT 13:26:50 ON 31 MAY 2006

FILE 'HOME' ENTERED AT 13:26:55 ON 31 MAY 2006

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 13:27:05 ON 31 MAY 2006
SEA ALICYCLOBACILLUS

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L1 QUE ALICYCLOBACILLUS

FILE 'CAPLUS, SCISEARCH, BIOSIS, MEDLINE, ESBIOBASE, PASCAL, FROSTI, EMBASE' ENTERED AT 13:28:20 ON 31 MAY 2006

L2 166 S L1 AND (POLYPEPTIDE ISOLAT? OR PURIF?)

L3 86 DUP REM L2 (80 DUPLICATES REMOVED)

=> d 13 ibib ab 80-86

L3 ANSWER 80 OF 86 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on
STN

ACCESSION NUMBER: 1995:74690 SCISEARCH
THE GENUINE ARTICLE: QC616
TITLE: ZYMOMONAS-MOBILIS SQUALENE-HOPENE CYCLASE GENE (SHC) -
CLONING, DNA-SEQUENCE ANALYSIS, AND EXPRESSION IN
ESCHERICHIA-COLI
AUTHOR: REIPEN I G (Reprint); PORALLA K; SAHM H; SPRENGER G A
CORPORATE SOURCE: KFA JULICH GMBH, FORSCHUNGSZENTRUM, INST BIOTECHNOL 1,
D-52425 JULICH, GERMANY; UNIV TUBINGEN, INST BOT
MIKROBIOL, D-72076 TUBINGEN, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: MICROBIOLOGY-UK, (JAN 1995) Vol. 141, Part 1, pp. 155-161.
ISSN: 1350-0872.
PUBLISHER: SOC GENERAL MICROBIOLOGY, HARVEST HOUSE 62 LONDON ROAD,
READING, BERKS, ENGLAND RG1 5AS.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 38
ENTRY DATE: Entered STN: 1995
Last Updated on STN: 1995

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Using a DNA probe from the gene encoding squalene-hopene cyclase (SHC,
EC 5.4.99.-) from the Gram-positive bacterium **Alicyclobacillus**
acidocaldarius, we have cloned a 4.3 kb HindIII fragment of chromosomal
DNA from *Zymomonas mobilis*. An open reading frame of 1977 bp was detected
that could encode a protein of 658 amino acids with a calculated molecular
mass of 74077 Da. Under the control of lac or tac promoters, this gene,
shc, was expressed in *Escherichia coli* K12 strains and its product had
squalene-hopene cyclase activity. Sequence alignments with the A.
acidocaldarius SHC, the lanosterol cyclase of the yeast *Candida albicans*.
and the cycloartenol synthase of the plant *Arabidopsis thaliana* revealed
six highly conserved regions (mainly in the C-terminal part) of the
proteins. These regions contained the core motif Gln-X-X-X-Gly-X-Trp.

L3 ANSWER 81 OF 86 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on
STN DUPLICATE 19

ACCESSION NUMBER: 1995:149207 SCISEARCH
THE GENUINE ARTICLE: QH606
TITLE: **PURIFICATION, PROPERTIES AND STRUCTURAL ASPECTS**
OF A THERMOACIDOPHILIC ALPHA-AMYLASE FROM
ALICYCLOBACILLUS-ACIDOCALDARIUS ATCC-27009 -
INSIGHT INTO ACIDOSTABILITY OF PROTEINS
AUTHOR: SCHWERMANN B (Reprint); PFAU K; LILIENSIEK B; SCHLEYER M;
FISCHER T; BAKKER E P
CORPORATE SOURCE: UNIV OSNABRUCK, MIKROBIOL ABT, FACHBEREICH 5, D-49069
OSNABRUCK, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (15 DEC 1994) Vol. 226,
No. 3, pp. 981-991.
ISSN: 0014-2956.
PUBLISHER: BLACKWELL PUBLISHING LTD, 9600 GARSINGTON RD, OXFORD OX4
2DG, OXON, ENGLAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 66
ENTRY DATE: Entered STN: 1995
Last Updated on STN: 1995

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The alpha-amylase from the thermoacidophilic eubacterium

Alicyclobacillus (*Bacillus*) *acidocaldarius* ius strain ATCC 27009 was studied as an example of an acidophilic protein. The enzyme was **purified** from the culture fluid. On an SDS/polyacrylamide gel, the protein exhibited an apparent molecular mass of 160 kDa, which is approximately 15% higher than that predicted from the nucleotide sequence. The difference is due to the enzyme being a glycoprotein. Deglycosylation or synthesis of the enzyme in *Escherichia coli* gave a product with the mass expected for the mature protein. The amylase hydrolyzed starch at random and from the inside, and its main hydrolysis products were maltotriose and maltose. It also formed glucose from starch (by hydrolysing the intermediate product maltotetraose to glucose and maltotriose) and exhibited some pullulanase activity. The pH and temperature optima were pH 3 and 75 degrees C, respectively, characterizing the enzyme as being thermoacidophilic. Alignment of the sequence of the enzyme with that of its closests neutrophilic relatives and with that of alpha-1,4 or alpha-1,6 glycosidic-bond hydrolyzing enzymes of known three-dimensional structure showed that the acidophilic alpha-amylase contains approximately 30% less charged residues than do its closests relatives, that these residues are replaced by neutral polar residues, and that hot spots for these exchanges are likely to be located at the surface of the protein. Literature data show that similar effects are observed in three other acidophilic proteins. It is proposed that these proteins have adapted to the acidic environment by reducing the density of both positive and negative charges at their surface, that this effect circumvents electrostatic repulsion of charged groups at low pH, and thereby contributes to the acidostability of these proteins.

L3 ANSWER 82 OF 86 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 20
 ACCESSION NUMBER: 1994:157437 CAPLUS
 DOCUMENT NUMBER: 120:157437
 TITLE: Active site mapping of affinity-labeled rat
 oxidosqualene cyclase
 AUTHOR(S): Abe, Ikuro; Prestwich, Glenn D.
 CORPORATE SOURCE: Dep. Chem., Stony Brook, NY, 11794-3400, USA
 SOURCE: Journal of Biological Chemistry (1994), 269(2), 802-4
 CODEN: JBCHA3; ISSN: 0021-9258
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Rat liver oxidosqualene cyclase (OSC), a 78-kDa membrane-bound enzyme, was **purified** and labeled with the mechanism-based irreversible inhibitor, [3H]29-methylidene-2,3-oxidosqualene. A 6-kDa CNBr peptide was separated by Tricine SDS-PAGE and blotted to a polyvinylidene difluoride membrane. The sequence of the first 30 amino acids of this peptide were determined by Edman degradation and showed unexpectedly high similarity to the fungal OSC from *Candida albicans* (50% identity with Arg413-Val442) and to the bacterial squalene cyclase from **Alicyclobacillus** (formerly *Bacillus*) *acidocaldarius* (37% identity with Lys356-Leu385). Further, radioanal. clearly established that the two adjacent Asp residues in the highly conserved region (Asp-Asp-Thr-Ala-Glu-Ala or DDTAEA) were equally labeled by the irreversible inhibitor. This result provides the first information on the structural details of the active site of OSC and shows for the first time the ancient lineage of this vertebrate enzyme to ancestral eukaryotic and prokaryotic cyclases. Interestingly, the covalently modified DDXX(D/E) sequence of rat liver OSC showed surprising similarity to the putative allylic diphosphate binding site sequence of sesquiterpene cyclases and prenyl transferases.

L3 ANSWER 83 OF 86 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
 ACCESSION NUMBER: 1994:244859 SCISEARCH
 THE GENUINE ARTICLE: NF450
 TITLE: ENZYMATIC CYCLIZATION OF 2,3-DIHYDROSQUALENE AND SQUALENE
 2,3-EPOXIDE BY SQUALENE CYCLASES - FROM PENTACYCLIC TO

TETRACYCLIC TRITERPENES

AUTHOR: ABE I (Reprint); ROHMER M
 CORPORATE SOURCE: ECOLE NATL SUPER CHIM, F-68093 MULHOUSE, FRANCE
 COUNTRY OF AUTHOR: FRANCE
 SOURCE: JOURNAL OF THE CHEMICAL SOCIETY-PERKIN TRANSACTIONS 1, (7 APR 1994) No. 7, pp. 783-791.
 ISSN: 0300-922X.

PUBLISHER: ROYAL SOC CHEMISTRY, THOMAS GRAHAM HOUSE, SCIENCE PARK MILTON ROAD, CAMBRIDGE, CAMBS, ENGLAND CB4 4WF.

DOCUMENT TYPE: Article; Journal
 FILE SEGMENT: PHYS; LIFE
 LANGUAGE: English
 REFERENCE COUNT: 40
 ENTRY DATE: Entered STN: 1994
 Last Updated on STN: 1994

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Cell-free systems from the protozoon *Tetrahymena pyriformis* and the bacterium *Alicyclobacillus acidocaldarius* normally convert squalene into pentacyclic triterpenes of the gammacerane and hopane series. 2,3-Dihydrosqualene, a substrate analogue lacking one of the terminal double bonds of squalene and therefore making it impossible to form pentacyclic products, was converted unexpectedly into tetracyclic triterpenes, i.e. euph-7-ene by the *T. pyriformis* enzyme and a 1:1 mixture of (20R)-dammar-13(17)-ene and (20R)-dammar-12-ene by the system from *A. acidocaldarius*. Formation of a pentacyclic framework with six-membered D-ring might thus only depend on the assistance of the terminal double bond to the cyclization process, its lack of participation leading, probably spontaneously, to the formation of the thermodynamically favoured tetracyclic skeleton with a five-membered D-ring.

The cell-free system from *T. pyriformis* was further, for the first time, directly shown to induce cyclization of (3S)-[3-H-3]squalene epoxide into, gammacerane-3 beta,21 alpha-diol, and the (3R)-enantiomer into gammacerane-3 alpha,21 alpha-diol. The (3R)-enantiomer also afforded a novel monocyclic product with a 2,3,4-trimethylcyclohexanone structure. The enzymic cyclization of squalene epoxide is apparently exclusively initiated by an oxirane ring-opening, and not by a proton attack on the remaining terminal double bond of the molecule.

L3 ANSWER 84 OF 86 MEDLINE on STN DUPLICATE 21

ACCESSION NUMBER: 94075956 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 8254309
 TITLE: Cloning and sequencing of a gene encoding acidophilic amylase from *Bacillus acidocaldarius*.

AUTHOR: Koivula T T; Hemila H; Pakkanen R; Sibakov M; Palva I
 CORPORATE SOURCE: Department of Genetics, University of Helsinki, Finland.
 SOURCE: Journal of general microbiology, (1993 Oct) Vol. 139, No. 10, pp. 2399-407.
 Journal code: 0375371. ISSN: 0022-1287.

PUB. COUNTRY: ENGLAND: United Kingdom
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-X62835
 ENTRY MONTH: 199401
 ENTRY DATE: Entered STN: 3 Feb 1994
 Last Updated on STN: 18 Dec 2002
 Entered Medline: 10 Jan 1994

AB Two starch-degrading enzymes produced by *Bacillus acidocaldarius* (renamed as *Alicyclobacillus acidocaldarius*) were identified. According to SDS-PAGE, the apparent molecular masses of the enzymes were 90 and 160 kDa. Eight peptide fragments and the N-terminal end of the 90 kDa polypeptide were sequenced. An oligonucleotide, based on the amino acid sequence of a peptide fragment of the 90 kDa protein, was used to screen a

lambda gt10 bank of *B. acidocaldarius*, and the region encoding the 90 kDa protein was cloned. Unexpectedly, the ORF continued upstream of the N terminus of the 90 kDa protein. The entire ORF was 1301 amino acids (aa) long (calculated molecular mass 140 kDa) and it was preceded by a putative ribosomal binding site and a promoter. Computer analysis showed that the 1301 aa protein was closely related to an alpha-amylase-pullulanase of *Clostridium thermohydrosulfuricum*. We suggest that the starch-degrading 160 kDa protein of *B. acidocaldarius* is an alpha-amylase-pullulanase, and the 90 kDa protein is a cleavage product of the 160 kDa protein. Another ORF, apparently in the same transcription unit, was found downstream from the amylase gene. It encoded a protein that was closely related to the maltose-binding protein of *Escherichia coli*.

L3 ANSWER 85 OF 86 PASCAL COPYRIGHT 2006 INIST-CNRS. ALL RIGHTS RESERVED.
on STN

ACCESSION NUMBER: 1994-0100126 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 1994 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): Cloning and sequencing of a gene encoding acidophilic amylase from *Bacillus acidocaldarius*
AUTHOR: KOIVULA T. T.; HEMILAE H.; PAKKANEN R.; SIBAKOV M.; PALVA I.
CORPORATE SOURCE: Univ. Helsinki, dep. genetics, Helsinki, Finland
SOURCE: JGM. Journal of general microbiology, (1993), 139(p.10), 2399-2407, 35 refs.
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United Kingdom
LANGUAGE: English
AVAILABILITY: INIST-4410, 354000048267240120

AB Two starch-degrading enzymes produced by *Bacillus acidocaldarius* (renamed as **Alicyclobacillus** *acidocaldarius*) were identified. According to SDS-PAGE, the apparent molecular masses of the enzymes were 90 and 160 kDa. Eight peptide fragments and the N-terminal end of the 90 kDa polypeptide were sequenced. An oligonucleotide, based on the amino acid sequence of a peptide fragment of the 90 kDa protein, was used to screen a λ gt10 bank of *B. acidocaldarius*, and the region encoding the 90 kDa protein was cloned. Unexpectedly, the ORF continued upstream of the N terminus of the 90 kDa protein. The entire ORF was 1301 amino acids (aa) long (calculated molecular mass 140 kDa) and it was preceded by a putative ribosomal binding site and a promoter

L3 ANSWER 86 OF 86 MEDLINE on STN

ACCESSION NUMBER: 92256172 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1374624
TITLE: Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, **Alicyclobacillus** gen. nov.
AUTHOR: Wisotzkey J D; Jurtshuk P Jr; Fox G E; Deinhard G; Poralla K
CORPORATE SOURCE: Department of Biology, University of Houston, Texas 77204-5934.
SOURCE: International journal of systematic bacteriology, (1992 Apr) Vol. 42, No. 2, pp. 263-9.
Journal code: 0042143. ISSN: 0020-7713.
(Investigators: Fox G E, U Houston, TX)
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Space Life Sciences
OTHER SOURCE: GENBANK-X59163; GENBANK-X59164; GENBANK-X59165; GENBANK-X59166; GENBANK-X59167; GENBANK-X59168;

GENBANK-X59169; GENBANK-X60741; GENBANK-X60742;
GENBANK-X60743

ENTRY MONTH: 199206

ENTRY DATE: Entered STN: 26 Jun 1992
Last Updated on STN: 4 Sep 2002
Entered Medline: 12 Jun 1992

AB Comparative 16S rRNA (rDNA) sequence analyses performed on the thermophilic *Bacillus* species *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* revealed that these organisms are sufficiently different from the traditional *Bacillus* species to warrant reclassification in a new genus, **Alicyclobacillus** gen. nov. An analysis of 16S rRNA sequences established that these three thermoacidophiles cluster in a group that differs markedly from both the obligately thermophilic organisms *Bacillus stearothermophilus* and the facultatively thermophilic organism *Bacillus coagulans*, as well as many other common mesophilic and thermophilic *Bacillus* species. The thermoacidophilic *Bacillus* species *B. acidocaldarius*, *B. acidoterrestris*, and *B. cycloheptanicus* also are unique in that they possess omega-allylic fatty acid as the major natural membranous lipid component, which is a rare phenotype that has not been found in any other *Bacillus* species characterized to date. This phenotype, along with the 16S rRNA sequence data, suggests that these thermoacidophiles are biochemically and genetically unique and supports the proposal that they should be reclassified in the new genus **Alicyclobacillus**.

ACCESSION NUMBER: 1995:149207 SCISEARCH
THE GENUINE ARTICLE: QH606
TITLE: **PURIFICATION, PROPERTIES AND STRUCTURAL ASPECTS
OF A THERMOACIDOPHILIC ALPHA-AMYLASE FROM
ALICYCLOBACILLUS-ACIDOCALDARIUS ATCC-27009 -
INSIGHT INTO ACIDOSTABILITY OF PROTEINS**
AUTHOR: SCHWERMANN B (Reprint); PFAU K; LILIENSIEK B; SCHLEYER M;
FISCHER T; BAKKER E P
CORPORATE SOURCE: UNIV OSNABRUCK, MIKROBIOL ABT, FACHBEREICH 5, D-49069
OSNABRUCK, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (15 DEC 1994) Vol. 226,
No. 3, pp. 981-991.
ISSN: 0014-2956.
PUBLISHER: BLACKWELL PUBLISHING LTD, 9600 GARSINGTON RD, OXFORD OX4
2DG, OXON, ENGLAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 66
ENTRY DATE: Entered STN: 1995
Last Updated on STN: 1995

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The alpha-amylase from the thermoacidophilic eubacterium
Alicyclobacillus (*Bacillus*) *acidocaldarius* ius strain ATCC 27009
was studied as an example of an acidophilic protein. The enzyme was
purified from the culture fluid. On an SDS/polyacrylamide gel,
the protein exhibited an apparent molecular mass of 160 kDa, which is
approximately 15% higher than that predicted from the nucleotide sequence.
The difference is due to the enzyme being a glycoprotein. Deglycosylation
or synthesis of the enzyme in *Escherichia coli* gave a product with the
mass expected for the mature protein. The amylase hydrolyzed starch at
random and from the inside, and its main hydrolysis products were
maltotriose and maltose. It also formed glucose from starch (by
hydrolysing the intermediate product maltotetraose to glucose and
maltotriose) and exhibited some pullulanase activity. The pH and
temperature optima were pH 3 and 75 degrees C, respectively,
characterizing the enzyme as being thermoacidophilic. Alignment of the
sequence of the enzyme with that of its closests neutrophilic relatives
and with that of alpha-1,4 or alpha-1,6 glycosidic-bond hydrolyzing
enzymes of known three-dimensional structure showed that the acidophilic
alpha-amylase contains approximately 30% less charged residues than do its
closests relatives, that these residues are replaced by neutral polar
residues, and that hot spots for these exchanges are likely to be located
at the surface of the protein. Literature data show that similar effects
are observed in three other acidophilic proteins. It is proposed that
these proteins have adapted to the acidic environment by reducing the
density of both positive and negative charges at their surface, that this
effect circumvents electrostatic repulsion of charged groups at low pH,
and thereby contributes to the acidostability of these proteins.